# FINAL REPORT

19980229

**A STUDY OF THE EFFECTS OF CANADIAN VELVET ANTLER (CVA) ON WOUND HEALING**

**September, 2003**
**Funded by the Agri-Food Innovation Fund**

**Prepared by: University of Saskatchewan**

AGRI-FOOD INNOVATION FUND

Canada

Saskatchewan

106-01221/

# Effect of Oral and Topical Elk Antler Velvet Administration on Cutaneous Wound Healing in an Animal Model of Streptozotocin-Induced Diabetes Mellitus


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## PROJECT SUMMARY:

This study examined the effect of Elk Velvet Antler (EVA) in an animal model of diabetes mellitus. The cause of abnormal wound healing in diabetics is not known but deficient growth factor activity appears to play an important role. EVA has been reported to contain growth factor-like activity. This study examined the effect of oral and topical EVA administration by measuring skin wound closure on a daily basis, as well as growth factor content and histology. Daily topical application of water-soluble extracts of EVA decreased wound inflammation and increased the rate of wound closure when compared to control treatment. However, no differences in growth factor content were observed. Oral EVA administration failed to affect wound healing. Topical EVA treatment appears to improve diabetic wound healing however the active agent(s) and optimum treatment regimen need to be determined.

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## ABSTRACT:

An economical and effective agent to encourage repair in a patient population with compromised wound healing has yet to be discovered. Although several groups have reported some efficacy of Elk Velvet Antler (EVA) to augment healing, no published scientific data substantiates its efficacy in promoting the repair of compromised wounds. Our study is the first to examine the effect of both topically and orally administered EVA in a model of delayed wound healing (streptozotocin [STZ] - induced type-1 diabetes mellitus). In part one of the study, animals were separated into three groups receiving either a control diet, a bone meal supplemented diet or an EVA supplemented diet. Part two of the study involved two groups of animals with experimentally induced wounds treated with either a control topical gel or a topical gel containing water soluble extracts of EVA. Wound healing was assessed by measuring daily wound contraction, and by histological and growth factor (basic fibroblast growth factor and transforming growth factor-beta) analyses of wound biopsies taken 2 and 10 days postoperatively. No significant differences in contraction rate or biopsy analyses were observed between control, bone meal and EVA diets in the first part of the study. In the second part, topical administration of EVA-containing gel resulted in a trend towards an overall increase in the rate of wound healing, with significant increases 6 and 7 days following wounding. Although no significant differences were observed in wound growth factor content, EVA-treated animals did show a decrease in the extent of wound angiogenesis. This study indicates that topical administration of EVA may increase the rate wound healing. Further studies are warranted to determine whether EVA represents an economically viable alternative to the use of recombinant growth factors in delayed wound repair.

## 1. INTRODUCTION:

Cutaneous wound repair is a complex, interactive process involving three overlapping phases: inflammation, cellular proliferation and tissue remodeling.[1,2,3] Repair is regulated by a cascade of inflammatory mediators including cytokines and growth factors (cytokines exerting primarily mitogenic effects). Although numerous growth factors are involved, transforming growth factor-beta (TGF-β) arguably exerts the greatest fibrogenic effect during repair.[1,2] Indeed, it favours extracellular matrix accumulation through chemoattractant, proliferative and synthetic influences on parenchymal cells, in particular, the fibroblast. Another growth factor of documented importance in wound repair is basic fibroblast growth factor (bFGF). Through its potent angiogenic capacity bFGF promotes the deposition of granulation tissue[3], and accelerates repair, as measured by increase in tensile strength.[4]

While these mediators are known to perform an essential role in repair, complex spatial and temporal interactions between cells, the matrix and the mediators exist and have yet to be wholly elucidated.[1] This may explain the mitigated success of several studies investigating the use of growth factors to accelerate repair of normal skin wounds.[5,6,7] Furthermore, since normal wound repair proceeds at a rapid rate, particularly in the laboratory animals used in these studies, it is often difficult to confidently establish an improvement in the rate and / or quality of repair.[5,6]

Conversely, wound healing in patients with underlying metabolic disorders such as the diabetic, the elderly, or the severely burned, is fraught with complications. For example, in both experimental and clinical diabetic wound healing studies, compromises in cellular migration and proliferation, vascular proliferation and extracellular matrix remodeling have been reported.[8,9] These phenomenon lead to a decrease in granulation tissue and reduced amounts of collagen, protein, and DNA, with an overall effect of a

delay in wound healing and diminished tensile strength.[8,10] The underlying cause of delayed and aberrant wound healing has yet to be established, but deficient growth factor activity within the wound environment may play an important role.[11] Indeed, studies performed on animal models of impaired healing suggest that the addition of exogenous growth factors substantially promotes healing, and achieves rates of repair comparable with those of unimpaired, control animals.[12,13] In particular, it has been shown that both bFGF and TGF-β induce significant increases in the accumulation of granulation tissue in diabetic rat wounds.[8]

Extrapolating from United States data, approximately 1 million Canadians per year suffer from burns or chronic wounds.[14,15] Presently, only one recombinant growth factor product (becaplermin; platelet derived growth factor, PDGF) is approved for use in Canada in the treatment of diabetic foot ulcers.[16,17] The cost of treatment is currently prohibitive to most patients (approximately $1300 / ulcer).[18] Consequently, the development of an affordable and efficacious growth factor-based therapy would represent a major advancement in the treatment of compromised wounds.

Elk velvet antler (EVA) has been used in traditional Chinese medicine for over 2000 years as a preventative agent in a wide range of ailments. Of particular interest, EVA has been reported to enhance wound healing[19] Recent studies have shown that water soluble extracts of EVA contain growth factors which can stimulate fibroblast growth *in vitro*.[20] These noncharacterized growth factors could be responsible for the reported improvement of healing following oral administration of EVA.[19] The mechanism of action of orally delivered EVA is unknown but could result from substances found in EVA such as growth factors, glycosaminoglycans, proteoglycans or fatty acid/phospholipids.[21,22]

Our study is the first to critically examine the effects of oral and topical administration of EVA in an animal model of compromised wound healing. We hypothesized that EVA, either orally or topically administered, would accelerate dermal wound repair in diabetic rats. Furthermore, we predicted that enhanced repair would correlate with increased wound tissue concentrations of TGF-$\beta_1$ and bFGF.

## 2. METHODS:

### *2.1 Diabetes Mellitus (Type 1) Model[23,24]:*

Individually caged Sprague-Dawley rats (9 weeks old, 150-200g) were fasted for 24 hours before receiving of a single tail vein injection of streptozotocin (STZ, 55 mg/kg) in citrate buffer (pH 4.5), 30 days prior to wounding. STZ selectively destroys pancreatic beta-cells leading to metabolic, cardiovascular and wound healing abnormalities similar to those observed in humans with Type 1 (insulin-dependent) diabetes mellitus.[25] Buprenorphine (0.03 mg/kg) was injected i.m. 30 minutes prior to STZ for prophylactic analgesia. Hyperglycemia was confirmed after one week by the glucose oxidase method (One Touch, Lifescan), then glucose concentrations were measured at the time of wounding and at the end of the study. Food consumption and body weight were also monitored throughout the study.

All animal handling and experimental procedures were approved and carried out in accordance to the guidelines established by the **University of Saskatchewan's Committee on Animal Care and Supply** (UCACS).

### *2.2 Treatment Groups:*

2.2a) Oral Administration Study: Animals were randomly selected to receive one of the three treatment diets: control diet (standard rat feed), bone meal diet (10 g

bone meal per kg feed) and EVA diet (10 g of EVA per kg of feed). Each group consisted of 3 animals. Treatment diets were started 23 days after STZ injection and 7 days prior to wounding.

2.2b) Topical Administration Study: Animals were randomly selected to receive either 500 µl/wound/day of control gel (Muko Jelly) or EVA gel (water-soluble extract of EVA mixed with Muko Jelly). To obtain the water-soluble extract, 1.5 g of EVA was homogenized in 15 ml of deionized water (4°C). Homogenate was centrifuged at 40 000 X g for 30 min (4°C) and the resultant supernatant was collected and sterilized through 0.22-µm filters. Daily aliquots were stored at -80°C and thawed prior to use. Protein content of the supernatant was approximately 7.0 mg/ml as determined by Lowry protein method. A volume of extract containing 400 µg of protein was mixed with 500 µl of gel immediately prior to wound application.

### *2.3 Excisional Wounding Procedure:*

Rats were injected with buprenorphine (0.03 mg/kg, i.m.) 30 minutes before surgery. Anesthesia was induced and maintained with isoflurane. The hair on the back of each animal was clipped with electric clippers and cleaned with povidone-iodine cleansing solution. Two separate skin areas were outlined on either side of midline using a 10mm diameter circular template. Wounds were left unbandaged, as their inaccessible location prevented rats from licking the wounds after surgery or following the application of the topical gel.

Full thickness wounds were made by excising the marked area of skin to the level of subcutaneous tissue. Dissections were performed with a scalpel and iridectomy scissors to ensure that the wound edge was sharply defined and not contused.

Experimental or control diets were started one week prior to the time of wounding in the oral administration study, while topical gel was applied immediately following surgery and then once daily to the wounds of rats in the topical administration study. Wounds were left unbandaged and healed by second intention.

### *2.4 Assessment of Wound Healing:*

#### 2.4.1 Rate of Wound Closure:

One wound per animal was photographed daily alongside a landmark template until biopsy of that wound at the end of the study (10 days). Photos were taken under candescent (100 W) light using a Nikon CoolPix 990 digital camera on macro setting at maximum magnification and at a fixed distance of 25 cm. Digital images of each wound were visually scored using a mm grid and graphed as Percentage Wound Area per day and Log Percentage Wound Area per day based on the wound size one day after the surgery. Statistical analysis of daily wound closure was performed using a one-factor ANOVA with the Fisher's exact test. Rate of wound closure was determined by the slope *(k)* following linear regression of Log Percentage Wound Area per day. The three *k* values obtained from the oral study were compared by a one-factor ANOVA while a two tailed unpaired t-test was used for the two *k* values from the topical study.

#### 2.4.2 Biopsy Sample Preparation for Growth Factor and Histological Evaluation:

The photographed wound was biopsied at the end of the study (10 days), while the second wound on each animal was biopsied at 48 hours. Thirty minutes prior to the biopsy procedure, animals were given buprenorphine (0.03 mg/kg, i.m.) then induced and maintained in general anesthesia with isoflurane. Five mm full-thickness circular punch

biopsies were taken from the wound edge to include the original epithelial border, the new epithelium and granulation tissue. One half of the biopsy sample was prepared for histological examination while the other was prepared for measurement of growth factor content. Histological samples were immediately immersed in acid/alcohol/formaldehyde solution (5% acetic acid, 85% ethanol, 4% formaldehyde; 6% water). Samples for growth factor analysis were weighed and placed into 0.5 ml Dulbecco's Modified Eagle Medium supplemented with 10% fetal calf serum, then homogenized (Brinkman PT 10/35, 1450 rpm) and centrifuged (Beckman JA 18.1 rotor) at 30 000 Xg for 30 min to remove cellular material from the supernatant. Supernatants were filter-sterilised through 0.22 µm filters and frozen at -80°C, in individual 400 µl aliquots.

### 2.4.3 Histology:

Acid-alcohol formalin fixed tissues were processed to 6-µm paraffin sections and stained with either hematoxylin-eosin (HE) or Masson's trichrome stain (MT). Tissue sections were scored on a scale of 0 to 3 (none, mild, moderate, and marked) for the following parameters: acute inflammation (platelets, fibrin, polymorphonuclear cells [PMN]; mononuclear cells (MN) (macrophages, lymphocytes, plasma cells); angiogenesis (capillaries); fibrosis and collagen organization; epithelial coverage of the wound; and epithelial hyperplasia.[26] Fibrosis and collagen content were assessed using MT-stained tissues. A board certified veterinary pathologist performed all histological evaluations in a blind fashion. Mean values were compared by two tailed unpaired t-test.

### 2.4.4 Growth Factor Analyses:

Basic fibroblast growth factor (bFGF) and TGF-$\beta_1$ concentrations were measured using commercial human cytokine enzyme-linked immunosorbent assay (ELISA) kits (R

& D Systems, Inc., Minneapolis, MN). The TGF-$\beta_1$ assay is sensitive to 7 pg/ml and is TGF-$\beta_1$ specific. All samples for the TGF-$\beta_1$ assay were acid-activated. The bFGF assay has a sensitivity of 3 pg/ml. Both TGF-$\beta_1$ and bFGF have excellent cross-species reactivity (R & D Systems, Inc.).

DMEM containing 10% fetal bovine serum was used to determine the 0 pg/g tissue point of the standard curve, such that the amount of growth factor contained in the media was subtracted from all samples analyzed. Statistical difference was determined using a one-factor ANOVA at a significance level of 95% with the Fisher's exact test.

### *2.5 Statistical Analysis:*

Statistical analysis was conducted using Statview SE+ (1988), Abacus Concepts Inc.

## 3. RESULTS:

### *3.1 Oral Administration Study:*

#### 3.1.1 Blood Glucose Concentrations and Changes in Body Weight:

Throughout the experiment whole blood glucose levels were dramatically elevated compared to the reported normal rat levels of 3.7 to 4.2 mmol/L. One week after administration of STZ, blood glucose concentrations were not significantly different between test groups and were approximately 15.0 mmol/l. (Figure 1) Glucose concentrations continued to rise throughout the experiment however no significant differences were observed between test groups. A non-significant decrease in blood glucose concentration was observed in EVA treated animals at the time of wounding, due to an outlier. By the end of the study this animal's blood glucose concentration was similar to that of the other animals.

Figure 2 illustrates the mean percentage change in body weight for each test group. No significant differences were observed between test groups. All animals showed a substantial decrease in body weight following the 48 hour biopsy procedure, however symptoms of distress were observed in none of the animals. Weight loss appeared to be a response to the repeated use of anesthetic and analgesic agents. The blood glucose and body weight analyses showed that all animals were responding in a similar amnner to the STZ induction of diabetes mellitus.

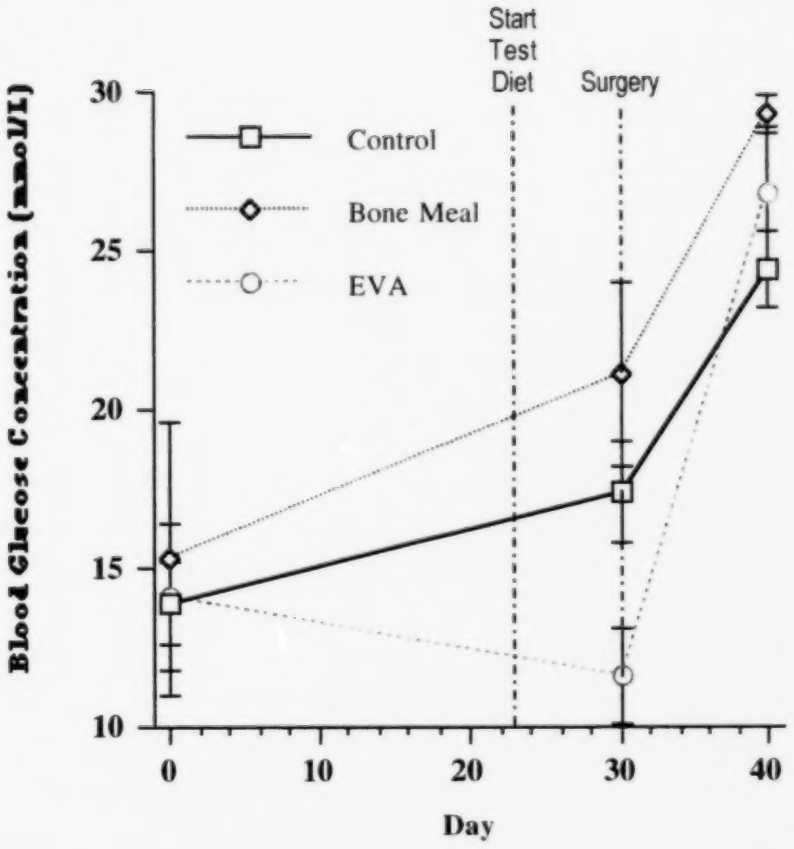



**Figure 1:** Average whole blood glucose concentrations for control, bone meal and EVA treated animals. Initial measurement, indicated as Day 0, was performed 2 weeks after STZ injection. Glucose was monitored at the time of surgery (Day 30) and at the end of the 10 day healing period (Day 40). Data points represent the mean±standard error (n=3) for each group.

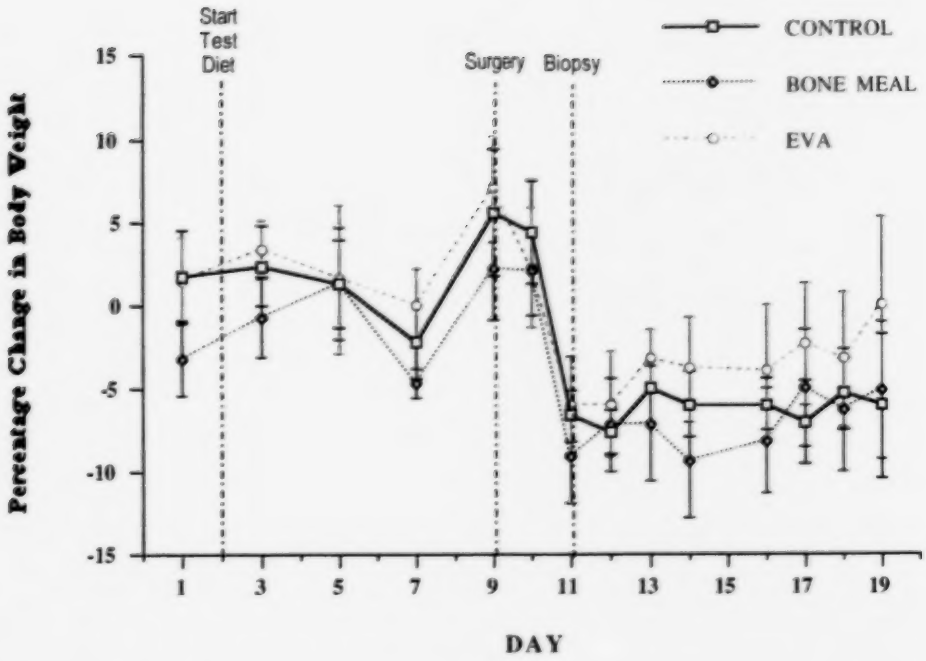



**Figure 2:** Mean values of percentage change in body weight from initial weight recording 4 days prior to initiation of test diets. Test diets started on Day 2, surgery conducted at Day 9 and first biopsy obtained at Day 11 are indicated by vertical dashed lines. Data points represent the mean±standard error (n=3) for each group.

### 3.1.2 Analysis of Wound Closure:

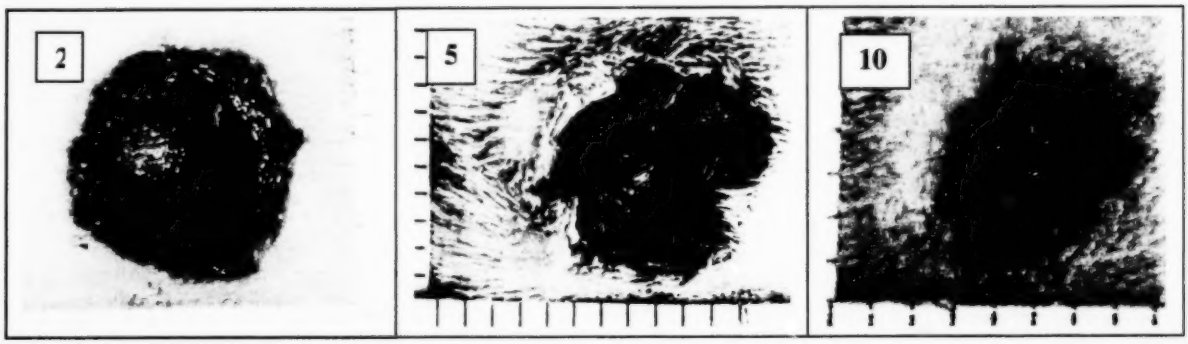
Figures 3a and 3b document wound closure in a control and EVA treated animal, respectively. No noticeable differences were observed in the rate of wound closure between control and treated of animals. Daily photographs were taken and the wound area quantified according to the 2 dimensional scale displayed beside the wound. Wound closure was graphed as the average percentage of original wound size versus time (day) post wounding (Figure 4a). To obtain a coefficient of wound closure, $k$, logarithm ($\log_{10}$) of the Percentage Wound Area on Day 1 was graphed versus time post wounding (Figure 4b). Linear regression of the mean data points for each treatment group yielded $k$ values of -0.081, -0.104 and -0.078 for control, bone meal and EVA groups respectively. Coefficients of healing were similar to those reported in other studies[19] and no significant differences were observed between groups.

### 3.1.3 Growth Factor and Histological Analyses:

There were no significant differences in TGF-β1 concentration between the three study groups at either 2 or 10 days post-surgery (Figure 5). We were unable to determine bFGF concentrations using the methodology described.

Histological assessment was compromised by the inherent difficulty of obtaining proper slide orientation to correctly visualise identifiable wound edges. Consequently, no statistical inferences could be made.

3a




3b

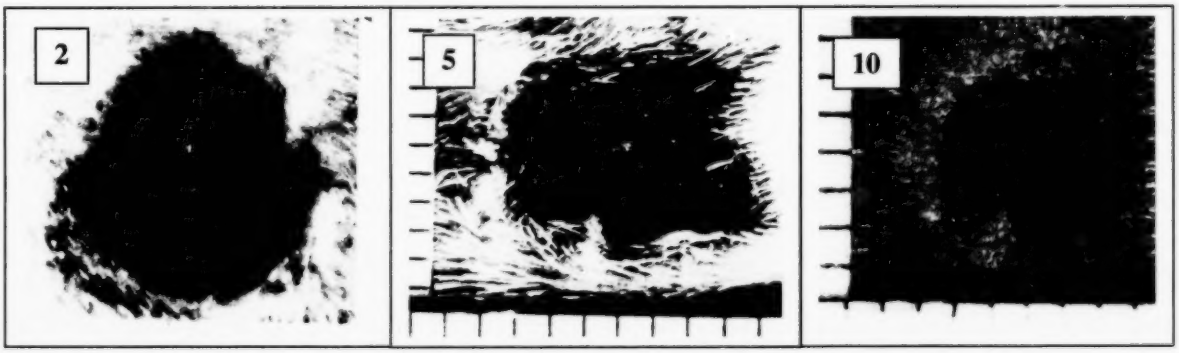



**Figure 3:** Wound photos taken from a control (3a) and a rat receiving EVA supplemented feed (3b). Numbers inset identify the day post wounding at which the photo was taken. Photos were taken under candescent light at a fixed distance of approximately 25 cm using a Nikon CoolPix 990 digital camera on macro setting at maximum magnification (2.5x).

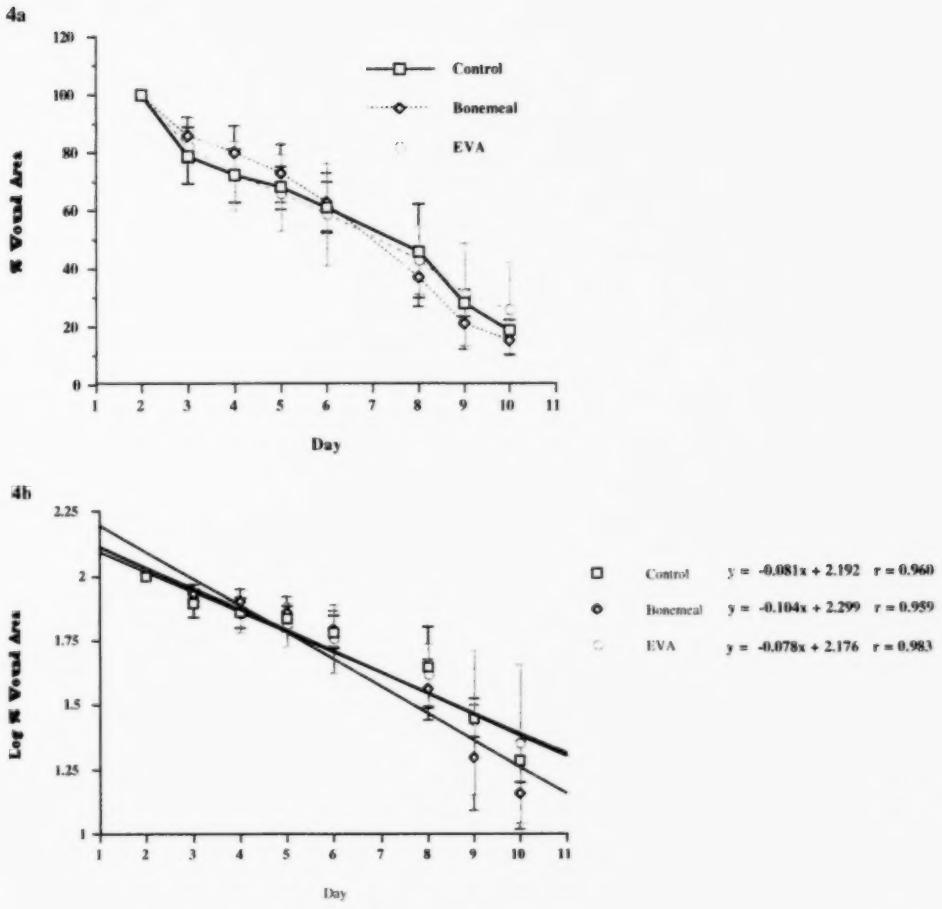



**Figure 4:** Figure 4a illustrates the Percentage of Wound Area versus time since wound surgery for each treatment group. Days represent days following initial wound surgery. Figure 4b illustrates the log percentage of Day 1 wound area versus time since wound surgery for each treatment group. The legend shows the linear regression values for each treatment group with the closure coefficient, *k*, being determined as the slope of the line. Data in both graphs are represented as the mean±standard error (n=3) for each group.

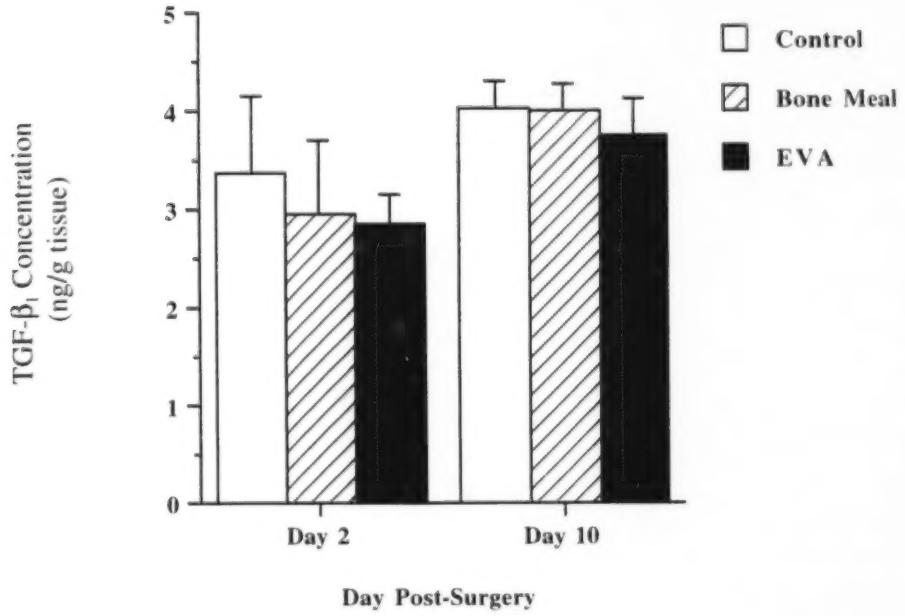



**Figure 5:** Concentration of acid-activated TGF-$\beta_1$ in wound tissues biopsied at 2 and 10 days post surgery from Oral study animals. Data points represent the mean±standard error (n=3) for each group.

### *3.2 Topical Administration Study:*

#### 3.2.1 Blood Glucose Concentrations and Changes in Body Weight:

Final blood glucose concentrations measured ten days after wounding showed no significant difference between animals receiving the control or EVA topical gel (Figure 6-II). Both groups are above 15 mmol/l, which is indicative of a diabetic state. Figure 6-I shows the average percentage body weight change in both groups; no significant difference was observed between groups.

#### 3.2.2 Analysis of Wound Closure:

Figures 7a and 7b illustrate the observed wound closure in a control and an EVA treated animal respectively. No noticeable abnormalities were observed in wounds throughout the study. Wounds were photographed and wound area quantified in the same manner as described in the oral administration study. Wound area measured post-operatively as a percentage of the Day 1 wound area is shown in Figure 8a. Wounds treated with EVA topical gel were significantly smaller by Days 6 and 7 of the study ($p<0.05$). Wound areas during the previous days, although consistently smaller in the EVA group, were not statistically different from those in the control gel group. The rates of wound closure assessed by the closure coefficient $k$ were $-0.058 \pm 0.007$ and $-0.076 \pm 0.010$ for control and EVA treated groups respectively (Figure 8b). Although these values were not statistically different, it appeared that EVA treatment increased the rate of wound closure.

### 3.2.3 Growth Factor and Histological Analyses:

Transforming growth factor-$\beta_1$ concentrations did not differ significantly between control and EVA topically treated animals at either 2 or 10 days post-surgery (Figure 9). We were unable to determine bFGF concentrations using the methodology described.

Histology of tissue biopsied two days after surgery showed diminished angiogenesis in wounds treated topically with EVA gel, as capillary content within these wounds was significantly decreased when compared to control topical gel treated wounds (Table 1). Although not statistically significant, a decrease in inflammation and an increase in fibrosis and collagen content were observed in EVA treated wounds when compared to control wounds.

**Table 1: Histological Evaluation of Topically Treated Wounds - 2 Days post Surgery**

| Histological Variable | Control | Topical EVA |
|---|---|---|
| Acute Inflammation (platelets, fibrin, PMN) | 1.2 ± 0.6 | 0.8 ± 0.4 |
| Mononuclear Cells (macrophages, lymphocytes, plasma cells) | 2.0 ± 0.4 | 1.2 ± 0.4 |
| Angiogenesis (capillaries) | 3.0 ± 0.0 | 1.6 ± 0.2* |
| Fibrosis and Collagen Content | 1.6 ± 0.2 | 2 ± 0.0 |

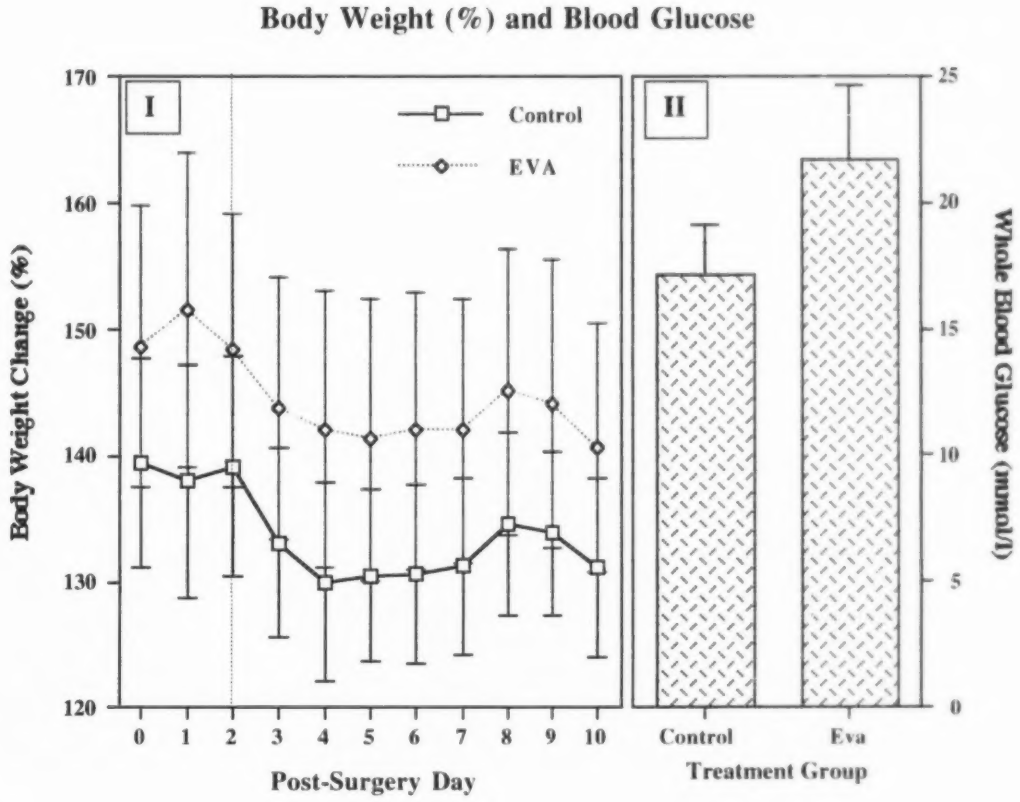
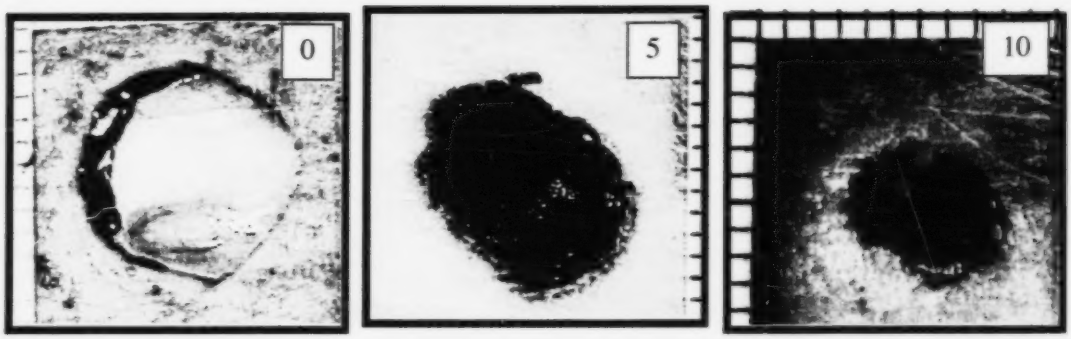



**Figure 6:** I. Mean values of percentage change in body weight with initial measurement made prior to injection of STZ. Surgery was conducted at Day 0 and first biopsy at Day 2 (indicated by vertical dashed line). Final biopsy was performed at Day 10 (not indicated). Data points represent the mean±standard error (n=5) for each group.

II. Average whole blood glucose concentration for control and EVA topically treated animals. Blood glucose was measured at the end of the 10 day healing period. Data points represent the mean±standard error (n=5).

7a




7b

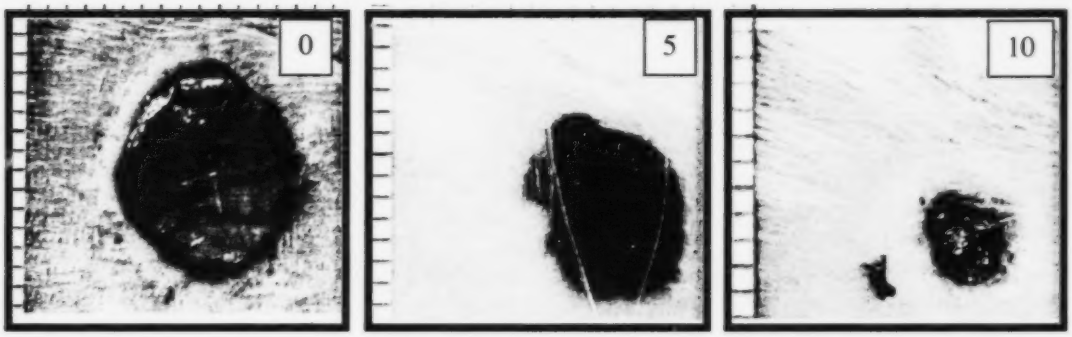



**Figure 7:** Wound photos taken from a control (7a) and an EVA-treated rat (7b). Numbers inset identify the post wounding day at which the photo was taken. Photos were taken under candescent light at a fixed distance of approximately 25 cm using a Nikon CoolPix 990 digital camera on macro setting at n aximum magnification (2.5x). Template marks are at mm intervals.

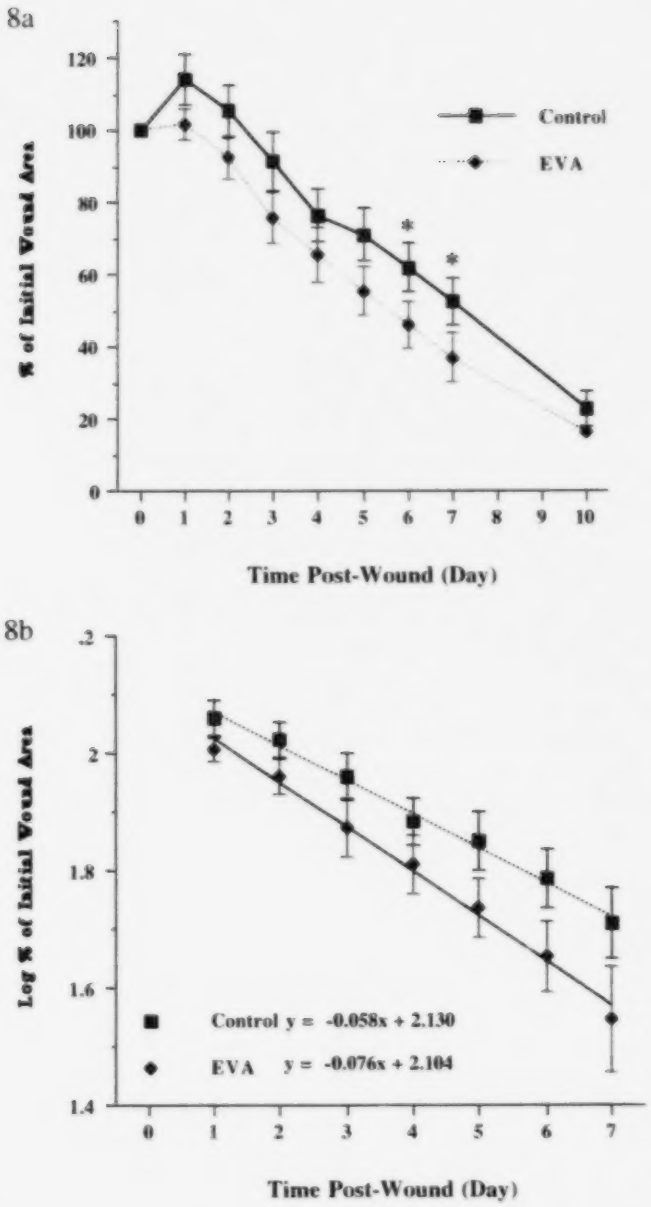



**Figure 8:** Figure 8a represents the percentage of wound area versus time for both treatment groups. Days represent days following initial wound creation. Figure 8b represents the log percentage of wound area versus time for each treatment group from day 1 to day 7. The legend shows the linear regression values for each treatment group with the closure coefficient, *k*, being determined as the slope of the line. Data in both graphs represent the mean±standard error (n=5) for each group. * indicates $p < 0.05$ compared to control.

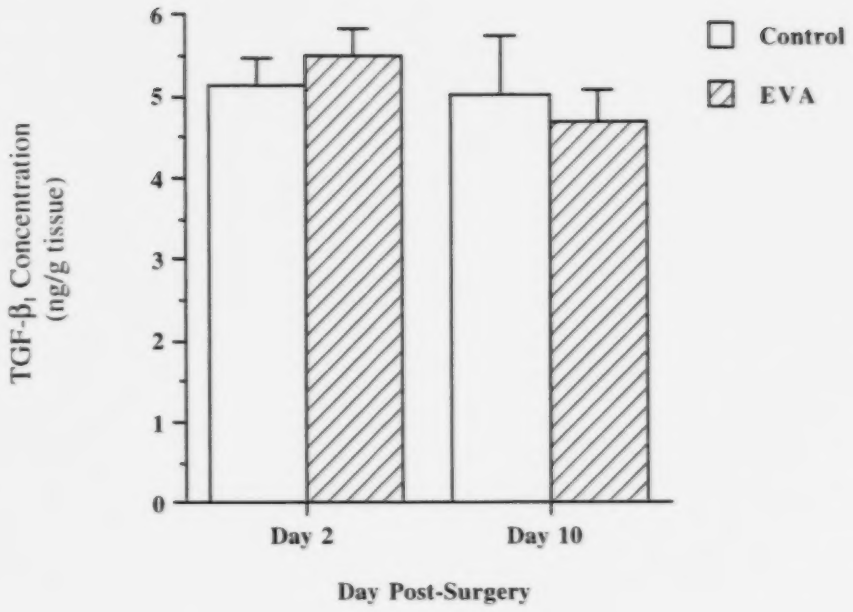



**Figure 9:** Concentration of acid-activated TGF-$\beta_1$ in wound tissue biopsied at 2 and 10 days post surgery from control topical gel and EVA-extract topical gel treated animals. Data points represent the mean±standard error (n=5) for each group.

## 4. DISCUSSION:

To the best of our knowledge, this is the first controlled study investigating the effects of orally and topically administered EVA on impaired wound healing *in vivo*. Results indicate that daily topical application of water-soluble EVA extract may accelerate secondary intention wound healing in diabetically impaired patients. Oral EVA treatment did not affect the rate of wound closure or any of the other measured parameters in this study.

Our rationale in selecting a model of compromised wound repair was two-fold. First, impaired wound healing represents an enormous burden on the healthcare system, both in terms of cost and the intensity of care required. Second, deficient wound healing systems are more sensitive targets for pharmacological manipulation. We chose an experimental model over a clinical trial since inherent variables present in the chronic wound environment and the myriad of underlying physiological imbalances suffered by affected patients limited our ability to find comparable populations of subjects with chronic wounds.

The diabetic model is particularly attractive for studying the effects of growth factors because the observed deficiency in granulation tissue formation is thought to result from impaired cellular entry as well as diminished angiogenesis.[27] It is hypothesized that diabetes interferes with the various stages of wound healing by decreasing growth factor levels in the wound environment.[11] Indeed, wound healing studies performed on streptozotocin induced diabetic rats have underlined the positive role of growth factors, such as bFGF and TGF-$\beta_1$.[8] Furthermore, a greater impact is achieved through combinations of growth factors over single formulations, given that each mediator exerts a principal effect on a particular phase of repair, and that a number of phases may be deficient in impaired healing situations.

In our quest to develop an effective yet inexpensive growth factor-based therapy to promote impaired wound healing, we have focused on a naturally occurring product: water soluble extract of EVA. Since it has been reported to stimulate dermal fibroblast growth *in vitro*,[20] we sought to investigate whether this action could be of benefit *in vivo*. Although daily topical application of EVA to excisional wounds in STZ induced diabetic rats grossly accelerated repair, we were unable to confirm this histologically. We did, however, note a negative effect of treatment on capillary content of the wound bed two days after surgery. This was unexpected, as we had hypothesized that growth factors present in EVA would reverse the inhibitory effect of STZ on angiogenesis. Moreover, it appears to contradict the enhanced rate of repair noted in EVA treated wounds. However, previous studies have reported no change in healing rates under conditions where angiogenesis was inhibited.[28,29,30] Trends observed in histological data indicate that EVA extract decreases wound inflammation, leading to an acceleration of the subsequent phases of repair, in particular deposition of collagen which is an important component of the mature extracellular matrix.

Since growth factor-like activity has been reported in velvet antler harvested from both elk[20] and deer,[31] and is credited for the rapid regenerative growth seen during the early phase of antler cycle and for acceleration of wound healing,[19] we speculated that concentrations of fibrogenic growth factors would be elevated in wounds treated topically with EVA. Following tissue injury TGF$\beta_1$ exerts chemoattractant and proliferative effects on fibroblasts,[1] and is believed to enhance wound contraction by stimulating fibroblast attachment and increasing the deposition of collagen matrix.[26,32] In addition to its chemoattractant properties, bFGF is mitogenic for both endothelial cells and fibroblasts, which favors granulation tissue formation and angiogenesis.[1,26,33] Results from our study suggest that acceleration of wound closure cannot be attributed to the

growth factors we chose to analyze, since neither TGFβ$_1$ nor bFGF differed between control and EVA treated animals.

Epidermal growth factor (EGF)-like and nerve growth factor (NGF)-like activities have been reported in deer antler studied *in vitro*.[31,34] In diabetic mice, human NGF increases the rate of cutaneous wound healing[35] while human EGF enhances oral wound healing.[36] Becaplermin (PDGF) is successfully used to accelerate dermal repair in diabetically compromised patients.[37] It is therefore possible that other growth factors are present in our water soluble EVA extract, and may be responsible for the observed acceleration in wound closure.

Finally, topical application of EVA extracts may positively influence repair by a mechanism unrelated to growth factor content. Indeed, it has been reported that EVA contains glycosaminoglycans and proteoglycans[38,39] which are important components of the extracellular matrix and thus play a role in wound healing.[40,41,42]

The lack of any positive effect on wound healing upon wound healing with oral administration of EVA is not unexpected. Aside from correcting the disturbed metabolic state present in type-II diabetes with either insulin or oral hypoglycemics or thiazolidinediones in type-II diabetes, an effective oral agent has yet to be established. Curcumin (diferuloylmethane) obtained from *Curcumin longa* has been reported to enhance wound repair in diabetic rats and mice with oral administration.[43] However, no reports have been published to confirm these findings. Another factor to be considered is the acute treatment regimen used in this study. EVA treated animals in this study were treated for only 7 days prior to wound surgery. Animals in this study were treated with oral EVA for only 7 days prior to wounding. Future studies should address these issues by altering the treatment regimen and including the use of type-II diabetic animal models as this condition would be more likely to respond to oral agents. Indeed, since EVA may

increase insulin release or receptor sensitivity[44]. EVA may correct the metabolic disturbances associated with type-II diabetes or may directly enhance insulin's effect on wound healing.[45]

## 5. CONCLUSIONS:

In conclusion, our study has shown that topical treatment with water soluble extracts of EVA positively influences the rate of secondary intention wound closure in STZ induced diabetic rats. Further studies are necessary to determine the active ingredients and to establish an optimum treatment regimen.

Although, oral administration of EVA failed to affect wound healing in this study, the use of a longer treatment regimen or the use of non-insulin dependent (type-II) diabetic models may yield positive results.

The dynamic field of growth factor research continues to provide hope to patients with compromised healing; these minute proteins are expected to overcome the inhibitory actions of a hostile wound healing environment. The major limitation to these expectations being the prohibitive cost of recombinant growth factor-based therapies, research focussing on economical pharmacological alternatives, such as naturally available substrates, is welcome.

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